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Occurrence of Enterohaemorrhagic *E. coli* in raw meat samples in Kerala

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Abstract

A study was carried out to investigate the occurrence of Enterohaemorrhagic *Escherichia coli* (EHEC) in raw beef, goat and buffalo meat samples collected from Kozhikode, Thrissur and Alappuzha districts of Kerala. Molecular characterisation of the isolates was carried for the detection of virulence associated genes stx1, stx2, eaeA and hlyA. The samples were collected during a period of August 2014 to April 2015 from abattoirs and markets located in different parts of the three districts. All the samples were subjected to isolation and identification of EHEC after pre-enrichment in Trypticase Soya broth. Escherichia coli characteristic colonies from EMB agar plates were streaked on to Sorbitol MacConkey's agar plates with cefixime-tellurite supplements (CT-SMAC agar) and 4-methylumbelliferyl -beta- D glucuronide (MUG EC O157) agar and incubated overnight at 37 OC for the isolation of EHEC. The occurrence of EHEC in beef was noticed in 4.08, 1.02 and 0.81 per cent from Kozhikode, Thrissur and Alappuzha districts respectively. Goat meat samples collected from Thrissur and Alappuzha showed the presence of EHEC in 1.41 and 0.89 per cent of samples, respectively. However, 9.1 per cent of buffalo meat samples collected from Thrissur district was found to be positive for EHEC. An overall occurrence of EHEC was found in 14 out of 112 beef samples, 6 out of 104 goat samples and 13 out of 100 buffalo meatsamples. Polymerised chain reaction revealed the presence of stx 1, stx2 and ,hly A gene in 24, 17 and 3 EHEC positive isolates whereas none of the isolates revealed the presence of eae A gene. The study shows the need for rigid food safety measures to be taken to combat the potential pathogenic effects of harmful bacteria throughout the production chain from production till consumption.

Keywords: EHEC, Molecular characterization, PCR, food safety measures.

Introduction

Food borne diseases are a major public health problem of growing concern throughout the world. Enterohaemorrhagic *E. coli* (EHEC) is one of the major food-borne zoonotic pathogen responsible for sporadic outbreaks of infection worldwide. Ruminants especially cattle are considered as the main reservoir of EHEC and the main source for contamination of the food supply. In addition, other ruminants such as sheep, goats and buffalo are considered as significant reservoirs. EHEC is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked meat products and raw milk. Contamination of meat with faecal material in the slaughtering process is the main transmission route of EHEC. Faecal contamination of water and other foods as well as cross-contamination during food preparation will also lead to infection. Fruits and vegetables contaminated with dung during irrigation, harvesting or processing are implicated in transmitting EHEC (Caprioli, 2005). Surface waters are often polluted through run-off from organic wastes applied to agricultural land and from direct faecal deposition (McGee, 2002). Because of the low infectious dose (1 to 100 CFU) of EHEC (Paton and Paton, 1998) faecal-oral transmission can easily occur in settings of poor hygiene and close contacts. Raw meat can harbour

harmful pathogenic EHEC organisms causing diarrhoea and systemic manifestations such as hemorrhagic colitis, hemolytic uremic syndrome (HUS). The virulence of EHEC strains is mainly associated with their ability to damage intestinal epithelial cells and produce shiga toxins *stx*1 and or *stx*2. In addition to shiga toxins, *eae A* gene that encodes for intimin and the *hly* A gene that encodes for hemolysin are other main virulence factors.

There is limited information regarding the occurrence of EHEC in ruminant meat in Kerala. This study was conducted to determine the occurrence of EHEC contamination in retail raw beef, goat and buffalo meat.

Materials and Methods

For the isolation and identification of EHEC organisms from raw meat, the method described by Meng et al. (2001) was used with some modifications. 112 beef samples, 104 goat meat and 100 buffalo meat samples of 100g each in sterile polythene bags were collected from retail shops. The samples were taken from rump and back region as these regions were found to be contaminated more through contact with intestinal contents and hide. The samples were collected during a period of August 2014 to April 2015 from abattoirs and markets located in different parts of three districts *viz.*, Thrissur, Kozhikode and Alappuzha of Kerala. The samples were collected aseptically in sterilized polythene bags and transported to the laboratory under chilled condition. The samples were processed upon arrival on the laboratory on the same day of collection.

The samples were homogenized in a stomacher for three minutes. From the homogenized sample 25 g was weighed and added to 225 ml of Trypticase Soya Broth (TSB) supplemented with Novobocin (20 mg/l) for pre enrichment and incubated for 24 h at 37^0C. After incubation 0.1 ml of the primary enriched broth were transferred to 10 ml of selective enrichment EC O157: H7 broth at 37 ^{0}C for 24 h. After incubation a loopful of the inoculum was plated on Cefixime Tellurite- Sorbitol Mac Conkey (CT- SMAC) agar and 4-methylumbelliferyl -beta- D- glucuronide (MUG EC O157) agar (Fujisawa et al. 2000) and incubated at 37 ^{0}C for 24 h. The colonies showing characteristics neutral grey green colonies with smoky centers on CT-SMAC agar were then subjected to primary and secondary biochemical identification test. Then the identification of virulence genes (*stx* 1, *stx* 2, *hly* A and *eae* A genes) was carried out using polymerase chain reaction (Louie et al., 1994).

Results

The occurrence of EHEC in beef was noticed in 4.08, 1.02 and 0.81 per cent from Kozhikode, Thrissur and Alappuzha districts respectively. Table.1. shows the overall occurrence of EHEC in 14 out of 112 beef samples, 6 out of 104 goat samples and 13 out of 100 buffalo meat samples.

Eight samples from Kozhikode three samples each from Thrissur and Alappuzha out of 51, 34 and 27 samples respectively showed the presence of EHEC. *Stx*1and *stx*2 gene was identified in eight EHEC isolates each from beef samples. Three of the isolates carried *hly* A gene. Goat meat samples collected from Thrissur and Alappuzha showed the presence of EHEC in 1.41 and 0.89 per cent of samples, respectively. Out of 47 and 29 goat meat samples collected from Thrissur and Alappuzha respectively, three samples each showed the presence of EHEC. *Stx*1 and *stx*2 gene was identified in all the EHEC positive isolates. However, 9.1 per cent of buffalo meat samples collected from Thrissur district was found to be positive for EHEC. EHEC was isolated from 13 buffalo meat samples out of 70 samples collected from Thrissur. 10 and three EHEC isolates carried *stx* 1 and *stx2* genes, respectively. Polymerised chain reaction revealed (Table. 1) the presence of *stx* 1, *stx*2 and, *hly* A gene respectively in 24, 17 and 3 EHEC positive isolates whereas none of the isolates revealed the presence of *eae* A gene. The study shows the need for rigid food safety measures to be taken to combat the potential pathogenic effects of harmful bacteria throughout the production chain from production till consumption

Table.1 Occurrence of EHEC and presence of virulent genes in positive isolates in raw meat samples

Sl No.	Sources	Positive samples (samples collected)	Genes identified			
			***Stx*1**	***Stx*2**	***Eae* A**	***Hly* A**
1.	Beef	14 (112)	8	8		3
2.	Goat meat	6 (104)	6	6	-	-
3.	Buffalo meat	13 (100)	10	3	-	-

Discussion

Of the 112 fresh beef samples screened, the prevalence rate of EHEC was 15.68 per cent. The present study revealed that the occurrence of EHEC in beef is in accordance with the results of Bekele *et al*. (2014) and Lindqvist *et al*. (1998) who had isolated EHEC from 15.5 per cent beef sample and Hessain *et al*. (2015) who had reported 2.97 per cent occurrence in beef samples. In the present study, 15.6 per cent positive beef samples were obtained from Kozhikode, followed by Thrissur (1.02 per cent), and Alappuzha (0.11 per cent). In the current study, the isolated EHEC isolates were characterized by the detection of shiga toxin type 1 and 2 (*stx*1 and *stx*2), *eae* and *hly*A genes by polymerised chain reaction. It is evident from the results obtained that specific shiga-like toxin genes (*stx*1 and *stx*2) were present in 24 (7.92 per cent) and 17(5.61 per cent) out of 33 EHEC isolates from beef, goat meat and buffalo meat samples whereas none of the isolates revealed the presence of *eae* A gene. The results are in accordance with Momtaz *et al*. (2013) who obtained more than one virulence gene including *Stx*1, *Stx*2, *eae* A and *hly* in all the EHEC isolates from raw meat samples.

Strategies to reduce EHEC in foods will depend much on hygienic and sanitary production and processing practices. This is to reduce the colonisation, transmission and cross contamination of EHEC in foods and the environment. An effective control measure for this pathogen has to target the farm, processing plants and the environments. At all these stages, strict adherence to standard operating measures must be practiced.

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